

Store at room temperature

### PRODUCT DESCRIPTION

LymphoSpinner is an innovative 50 mL separation tube with a porous barrier for optimal separation of lymphocytes and mononuclear cells from blood and tissues. The biologically inert barrier prevents mixing of the sample with the Lymphocyte Separation Medium (LSM™), thereby maintaining a discontinuous gradient and improving separation with higher yield and less processing time. This product is sterile and designed for single use only.

### ADDITIONAL REAGENTS & EQUIPMENT REQUIRED (NOT PROVIDED)

- ▶ Lymphocyte Separation Medium (LSM™, Cat. No. 0850494 or LymphoSep™, Cat. No. 091692249)
- ▶ Centrifuge (swinging bucket rotor) capable of generating 1,000 x g
- ▶ Centrifuge tubes for washing mononuclear cells
- ▶ Phosphate buffered saline solution (Cat. No. 091860454) or similar alternative cell culture medium

### PROCEDURE

Following the addition of lymphocyte separation medium to the LymphoSpinner Tube, a brief centrifugation places the density gradient medium below the frit. The blood sample can be added to the top chamber of the tube without risk of mixing with density gradient medium under the frit. After centrifugation, lymphocytes and other mononuclear cells remain at the plasma/density gradient medium interface. This dense band of mononuclear cells may be collected by pouring off the contents of the upper chamber or by means of a pipette. Erythrocyte contamination is avoided due to the barrier between the chambers.

- 1 Pipette 15 mL of room temperature Lymphocyte Separation Medium (LSM™, Cat. No. 0850494 or LymphoSep™, Cat. No. 091692249) into the upper chamber of the 50 mL tubes.

**NOTE ▶** Failure to bring the Lymphocyte Separation Medium to room temperature may cause a decrease in lymphocyte yield.

- 2 Centrifuge at 800 x g for 30 seconds at room temperature so the lymphocyte separation medium is below the frit.

- 3 Freely pour about 35 mL of diluted blood sample (defibrinated or heparinized human blood is diluted with physiological saline or balanced salt solution at a 1:1 ratio) into the upper chamber of the tube.

- 4 Centrifuge at 1,000 x g for 10 min or 800 x g for 15 minutes at room temperature.

**NOTE ▶** Centrifugation at lower temperatures, such as 4 °C, may result in cell clumping and poor recovery.

- 5 After centrifugation, carefully aspirate the plasma layer with a Pasteur pipette to within 2–3 mm of the opaque interface containing the mononuclear cells. Carefully transfer the opaque interface above the frit with a Pasteur pipette into a clean conical centrifuge tube.

- 6 Wash the cells by adding 10 mL of phosphate buffered saline or appropriate cell culture medium and mix the cells using a Pasteur pipette.

- 7 Centrifuge at 160–250 x g for 10 minutes at room temperature.

- 8 Aspirate the supernatant and discard.

- 9 Resuspend cell pellet with 5 mL of phosphate buffered saline or appropriate cell culture medium and mix the cells by using a Pasteur pipette.

- 10 Centrifuge at 160–250 x g for 10 minutes at room temperature.

- 11 Aspirate the supernatant and discard.

- 12 Repeat steps 9, 10 and 11, and resuspend cell pellet in appropriate medium for your applications.

